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THE SKELETAL ADAPTATION TO MECHANICAL USAGE IN THE RAT

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Abstract

The many similarities between the experimental findings in rats and Frost's concepts of skeletal adaptation to mechanical usage provide support for the rat unilateral, hindlimb bandaging model as a suitable model for underloading-induced bone loss and overloading-induced bone gain. Initially, conditions are characterized by underloading-induced transient bone loss due to depressed bone modeling-dependent bone gain and stimulated bone remodeling-dependent bone loss. Later, the bone loss stabilizes to a new steady state of rarefied bone with a lower bone turnover rate. Moreover, overloading-induced bone gain is caused by stimulated modeling-dependent bone gain and depressed bone remodeling-dependent bone loss. Furthermore, the rat unilateral hindlimb immobilization model using bandaging has other advantages: **a)** it is an inexpensive, reproducible model; **b)** dynamic histomorphometry can be readily performed; **c)** bone modeling (at least formation drift) and remodeling can be studied at different sites of the animal; **d)** both underloading and overloading responses can be studied in the same animal; **e)** it produces bone lost without or with a minimal regional acceleratory phenomenon; and **f)** reversibility can be readily studied. The disadvantages of the model are: **a)** biomechanical measurements of these small bones are limited and **b)** osteonal remodeling in normal rat cortical bone is lacking. These disadvantages are not important and if they are, we believe the advantages outweigh the disadvantages and we advocate the rat unilateral, hindlimb bandaging model as a suitable model for preclinical evaluation of new therapies for the prevention and reversal of bone loss due to immobilization.

Key Words: Rat, adaptation, osteopenia, negative and positive bone balance, bone modeling, bone remodeling, animal model, underloading, overloading.

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Introduction

The concept that bone tissue will adapt its structure according to load was developed more than a century ago. Despite the importance of this response, the mechanism and the structure-function relationship involved are still far from understood. Much of the work to date has dealt primarily with changes in underloaded bone. These changes include macroscopic changes in bone mass, cortical cross section and width, marrow cavity enlargement and trabecular volume (Smith and Gulligan, 1989). Unfortunately, the tissue organization and cellular mechanisms on which the mechanical adaptive responses are based have been ignored. Unless a greater number of well-designed mechanical usage studies are initiated, advances in this area of research will be limited.

Tissue Mechanisms Regulating Bone Mass

Mechanical usage can regulate changes in size and shape of bone and bone turnover. Processes which change size and shape of bones are referred to as modeling and processes involved with bone replacement mechanism are referred to as remodeling. The current concepts hold that during growth, modeling mechanisms shift cancellous and cortical bone surfaces. Modeling is a concept describing a combination of non-proximate, though coordinated resorption and formation drifts whose net result is to redistribute bone (Parfitt, 1990). Formation drift or formation associated with modeling (modeling in the formation mode) add new bone on the periosteal, corticoendosteal and trabecular surfaces. Formation drift is a process of forming bone without resorption (activation followed by formation). Resorption drift or resorption associated with modeling (modeling in the resorption mode) removes bone from these surfaces. (Resorption drift involve [activation, resorption and formation -ARF; see below]), with resorption greatly exceeding formation (Vignery and Baron, 1980). These drifts are called macromodeling in cortical bone and mini-modeling in cancellous bone (Frost 1988a,b, 1989). Macromodeling occurs mostly during growth and tends

to lessen in adult life (Frost, 1964; 1973; Parfitt 1983, 1990). However, trabecular mini-modeling can go on throughout life (Frost, 1990a).

Bone remodeling turns over bone. It is a tightly regulated process of bone resorption followed by bone formation. At the cellular level, the remodeling process begins by activating (A) resting cell populations on or near a bone surface. A stage of osteoclastic resorption (R) follows and removes a packet of bone called a resorption cavity. Bone formation (F) follows and fills the resorption cavity with new bone. ARF stands for the activation-resorption-formation sequence. At the cellular level, the collection of cells involved has been called a Basic Multicellular Unit (BMU) and the finished product is a Bone Structural Unit (BSU). Normally in man, it takes about 3 to 4 months and in the rat, it takes about 30 to 40 days to complete the ARF sequence or remodeling period (sigma). During one life span, more bone is reabsorbed than replaced leading to generalized bone remodeling dependent bone loss (Frost, 1986a,b; 1987; 1988a,b; 1990b; Kimmel and Jee, 1980; Parfitt, 1990).

In summary, remodeling usually results in a negative bone balance where bone resorption exceeds formation. The classical examples of a negative bone balance are age-related bone remodeling loss. However, there are some situations where the result is a positive bone balance in which bone formation exceeds resorption. An example is the net increase in bone mass induced by prostaglandin (Jee *et al.*, 1985, 1990; Li *et al.*, 1990b; Mori *et al.*, 1990).

Recent Developments

There have been two recent encouraging developments. One is Frost's global concept of skeletal adaptation to mechanical usage and the other is the use of a convenient, inexpensive small animal mechanical usage model that allows for histomorphometry analysis.

The principal stimulus for a more comprehensive approach to the tissue reactions of mechanically adaptive responses has been Frost's concepts of the mechanical usage effects on growth, modeling and remodeling (Frost, 1983a; 1986a,b; 1987; 1988a,b; 1990a,b). Frost summarizes these observations from clinical, experimental and histomorphometric evidence (1990b). Briefly, he states increased mechanical usage tends to increase bone mass during growth in three ways: it increases longitudinal growth that adds new spongiosa and cortex, it increases modeling drifts that add cortical cross-sectional area, and it suppresses the activation of new remodeling packets that reduce both the remodeling space and the net loss per completed packet. He further states disuse retards growth and modeling-dependent bone gain. Furthermore, disuse activates new remodeling packets. The

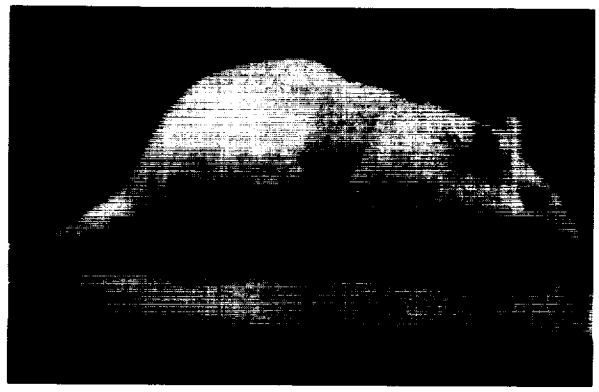

Fig. 1. A unilateral, hindlimb immobilized rat. The right hindlimb is immobilized against the abdomen by an elastic bandage with the hip joint in flexion and the knee and ankle joint in extension.

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gain in new packets allows for bone remodeling dependent bone loss. During adulthood, growth is absent and modeling drifts are minimal. Increased mechanical usage usually depresses bone remodeling dependent bone loss that retards loss of cancellous and subendocortical bone (reduces marrow cavity expansion). Acute disuse stimulates bone remodeling dependent bone loss, which accelerates expansion of the marrow cavity and loss of spongiosa.

The other breakthrough is the use of the rat, unilateral, hindlimb immobilization-induced osteopenia model (Li *et al.*, 1990a; Li and Jee, 1991; Jee and Li, 1990; Jee *et al.*, 1991) to study the cellular and tissue responses to mechanical usage. In general, one-legged immobilization by casting or bandaging is one of the better models for modifying mechanical loading because of its ease of application and its reversibility (Mattsson, 1972; Thomaides and Lindholm, 1976; Uhthoff and Jaworski, 1978; Jaworski 1986; Uhthoff *et al.* 1985; Jaworski and Uhthoff 1986). Several studies using this rat model have evaluated the hormonal effects and reversibility on disuse-induced osteopenia (Mattsson, 1972; Lindgren, 1976a,b; Lindgren and Mattsson, 1977). However, information on the dynamic histomorphometry on this model is missing. Unfortunately in the early 1970's, not many investigators employed today's dynamic histomorphometry technology to study the bone cell and tissue level mechanisms involved in load bearing because dynamic histomorphometry for cancellous bone was not devised by Frost until the mid-70's (Frost 1976, 1977; Frost *et al.*, 1981).

Recently, we have modified the Swedish adult rat hindlimb immobilization osteopenia model for mechanical usage studies (Li *et al.*, 1990a; Li and Jee, 1991; Jee and Li, 1990) employing modern histomorphometric

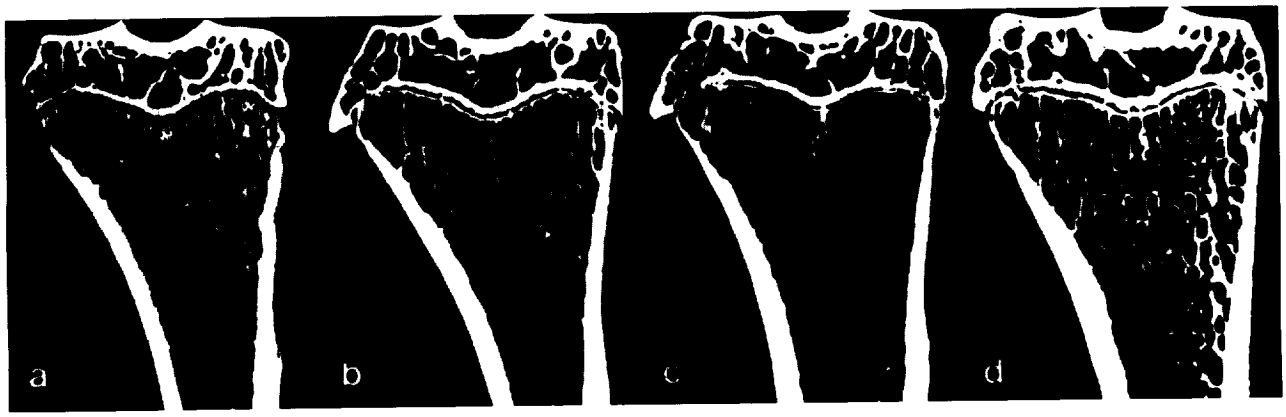

Fig. 2. Microradiographs of proximal tibiae from: **a)** a 9-month-old basal control, **b)** a 13.5-month-old after 18 weeks underloading and **d)** a 13.5-month-old after 18 weeks overloading. Fewer but thicker trabeculae are seen in the 13.5-month compared to the 9-month-old metaphysis (a vs. b). The underloaded tibia contains less cancellous tissue composed of fewer and thinner trabeculae (b vs. c). The overloaded tibia adds bone by conserving and thickening the trabeculae seen in the basal control (d vs. a).

techniques (Frost, 1969,1977; Frost *et al.*, 1981). We studied the time course of the effects of underloading and overloading bones to characterize changes in cancellous and cortical bone mass, architecture, tissue and cellular mechanisms in the double-fluorescent labeled unilateral, hindlimb immobilization adult rat. We started with 9-month-old virgin female rats. Changes in cancellous and cortical bone tissues induced by 2, 10, 18, and 26 weeks of underloading and overloading were compared to untreated, normally ambulating rats. To do so, we used the Lindgren (1976a) method of immobilization with some minor modifications. We anesthetized the rats, then bound the right hindlimb to the abdomen with four layers of elastic bandage tape (Elastikon, elastic tape, Johnson & Johnson, New Brunswick, NJ). In this position, the hip joint was in flexion while the knee and ankle joints were in extension. Since neither the right hindlimb nor the bandage touched the cage bottom during body movement, the left hindlimb carried the weight normally distributed between both hindlimbs (Fig. 1). One day after immobilization, the bandaged rats walked or hopped around on three limbs. The bandages were changed every week.

In the above experiment, we were able to (**a**) carry the experimental challenge without any discomfort to the animals, long enough to achieve steady states; (**b**) carry the experiment long enough so relatively small mechanical usage effects that could not be measured accurately after periods of a month or two, have sufficient time to become large enough to exceed the precision and errors of measurement; (**c**) consider the unimmobilized leg to be mildly overloaded instead of normal, unlike most others and provide proper controls which brought out the finding that this mild overloading led to increased accumulation of bone and (**d**) distinguish bone modeling from bone remodeling and designed the experiment to show the effects of mechanical underloading on these activities separately. A majority of others who have published animal experiments dealing with these problems have failed to do so.

Effects of Underloading and Overloading in 9-month-old rats

Effects of Underloading

Cancellous bone loss from the proximal tibial metaphysis occurred rapidly before 10 weeks and stabilized at 60% less bone than controls after 18 weeks of underloading (Fig. 2). The negative bone balance was caused by a combination of a transient elevated bone resorption at 2 and 10 weeks and depressed bone formation at 2 weeks (Li *et al.*, 1990a, Fig. 3). The new steady state after 18 weeks is characterized by rarified cancellous bone with a slightly lower turnover rate. The underloaded tibiae contained less cortical bone mass since nearly all periosteal bone formation had been blocked. At the same time, the marrow cavity enlarged (Li and Jee, 1991; Figs. 4 and 5). Furthermore, the bone remodeling cycle was prolonged to augment the negative bone balance (Akamine *et al.*, in press). We concluded that underloading stimulates cancellous and cortical remodeling dependent bone loss and depresses cortical bone modeling dependent bone gain. The bone remodeling dependent bone loss involves bone resorption exceeding bone formation and uncoupling bone formation from bone resorption. The modeling dependent bone

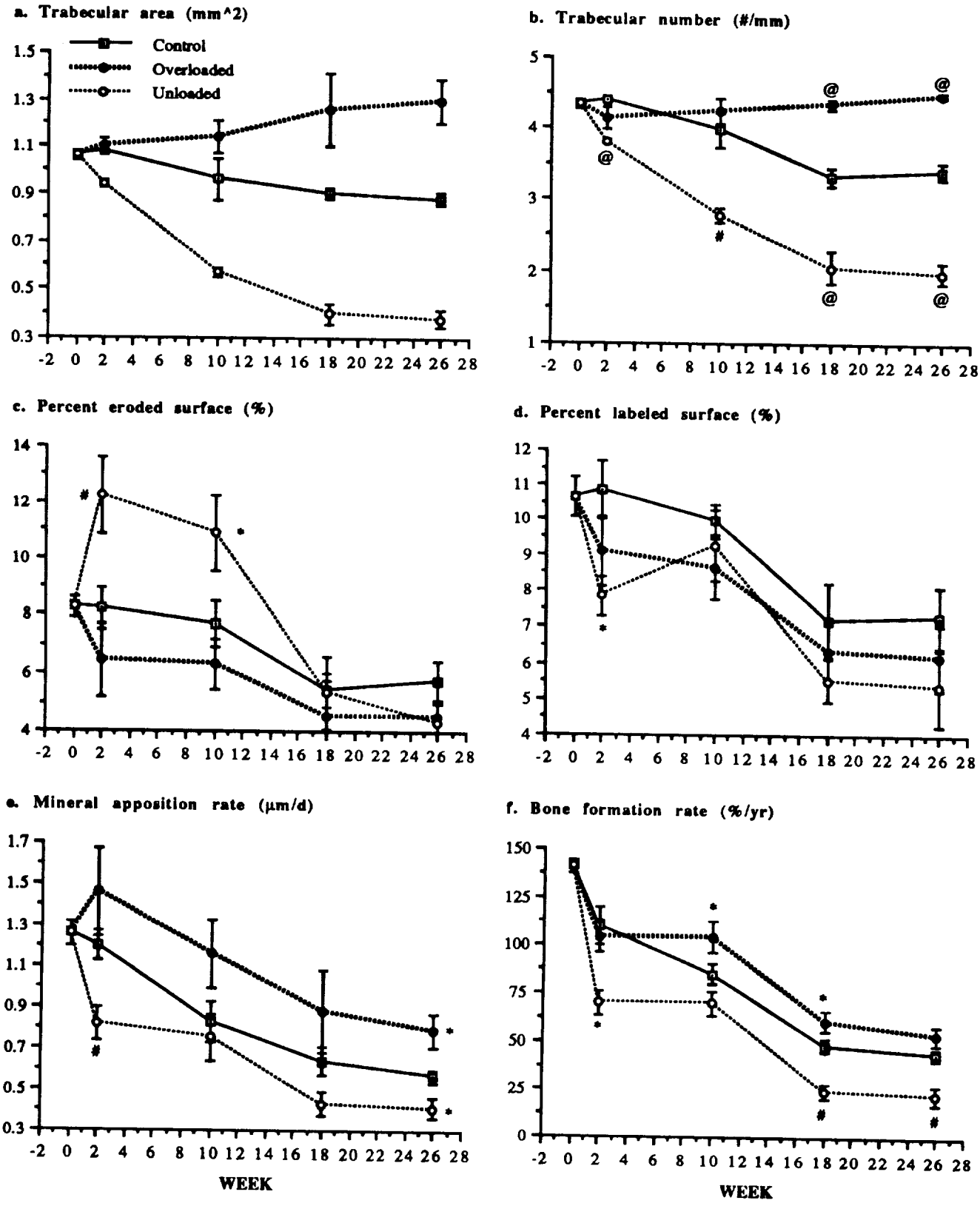

Fig. 3. Time course of select static and dynamic histomorphometry changes of the proximal tibial metaphyseal cancellous bone in control, underloaded and overloaded limbs. Y error bars represent standard errors. $^{*}p < 0.5$, $^{\#}p < 0.1$ and $^{@}p < 0.001$ from controls. See text for explanation. Modified from Li *et al.*, 1990 and Jee and Li, 1990.

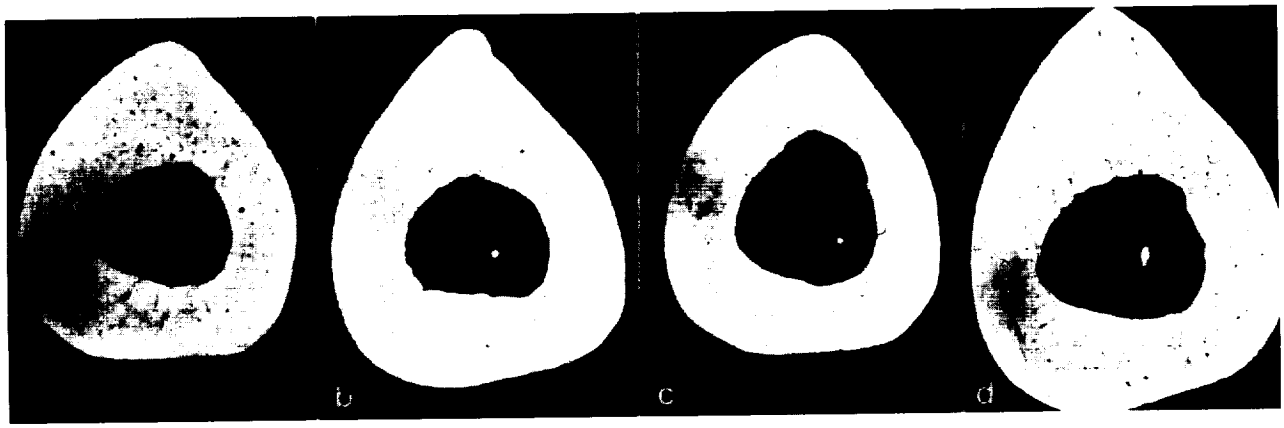

Fig. 4. Microradiographs of cross-sections of tibial diaphysis from: (a) beginning control; (b) 15-month-old aging control; (c) 15-month-old underloaded for 26 weeks; and (d) 15-month-old overloaded rats for 26 weeks. Note the difference in cross-sectional and marrow areas in the various cortices. The underloaded bone is smaller in the cross-sectional area and larger in the marrow area than aging control bone (c vs. b). Increased loading causes a larger cross-sectional area, but with the same size marrow cavity as in age-related controls (d vs. b).

loss involves a depression in formation drift. These findings are a refinement of Frost's concept of underloading since he stated that underloading increases bone remodeling-dependent bone loss and decreases bone modeling dependent bone gain.

Effects of Overloading

Overloading the proximal tibial metaphysis at first increased cancellous bone mass by 44% over that of controls and then stabilized after the 18th week (Fig. 2). This positive bone balance was accompanied by three changes: a significant increase in osteoblastic activity (mineral appositional rate) at 26 weeks, a significant increased bone formation rate at 10 and 18 weeks (Fig. 3; Jee and Li, 1990), and a significant decrease in the bone resorption period that did not alter the length of the bone remodeling period (Akamine *et al.*, personal communication). Overloading was effective in preventing age-related cortical bone loss, but not effective in adding bone. It creates a slight, positive cortical bone balance after 18 weeks by an insignificant increase in periosteal bone formation and a decrease in endocortical eroded surface (Fig. 5; Jee *et al.*, 1991). We concluded that overloading tends to stimulate formation associated bone modeling and remodeling; depresses the activation of resorption associated remodeling, and shortens the resorption period during bone remodeling. These findings again are in general agreement with Frost's concepts.

Bone Modeling and Remodeling in Older Rats

Currently we are convinced the secondary spongiosa of the tibial metaphysis remodels as well as models in these 9 to 15.5 month old rats. The proximal tibia is elongating slowly at 9 months (at less than 5 μm/d). The proximal tibial epiphyseal plate does not begin to close until 12 months. This means that at most 450 microns of new metaphyseal tissue is laid down between 9 and 12 months. The slow continued bone elongation means the proximal tibial periosteal envelope may still be modeling or capable of modeling and, if so, the secondary spongiosa may be minimodeling (that is, resorption and formation drifts are continuing on trabecular surfaces). Frost (1990a) has stated minimodeling does happen in adult skeleton and we have observed it in overloaded trabeculae in 15-month-old rats in the proximal tibial metaphysis (Jee and Li, 1990). However, we found there were very few such sites and most sites were remodeling. Therefore, the bulk of the secondary spongiosa is undergoing remodeling.

It is well established that in the rat, periosteal formation drift (formation associated modeling) is the manner in which subperiosteal bone is added to the tibial shaft throughout its life span (Kiebzak *et al.*, 1988a,b,c). Periosteal bone formation associated with modeling (formation drift) consists of activation followed by formation without intervening bone resorption. However, at the endocortical surface, we have concluded the bulk of this envelope is undergoing resorption associated bone remodeling. Periosteal bone formation associated with modeling (formation drift) and endocortical resorption associated with remodeling (resorption exceeding formation) is similar to what has been described for alveolar bone drifting in rats by Vignery and Baron (1980). Thus, the periosteal and endosteal bone sites we studied are involved with bone formation associated with modeling (formation drift) and bone resorption associated with

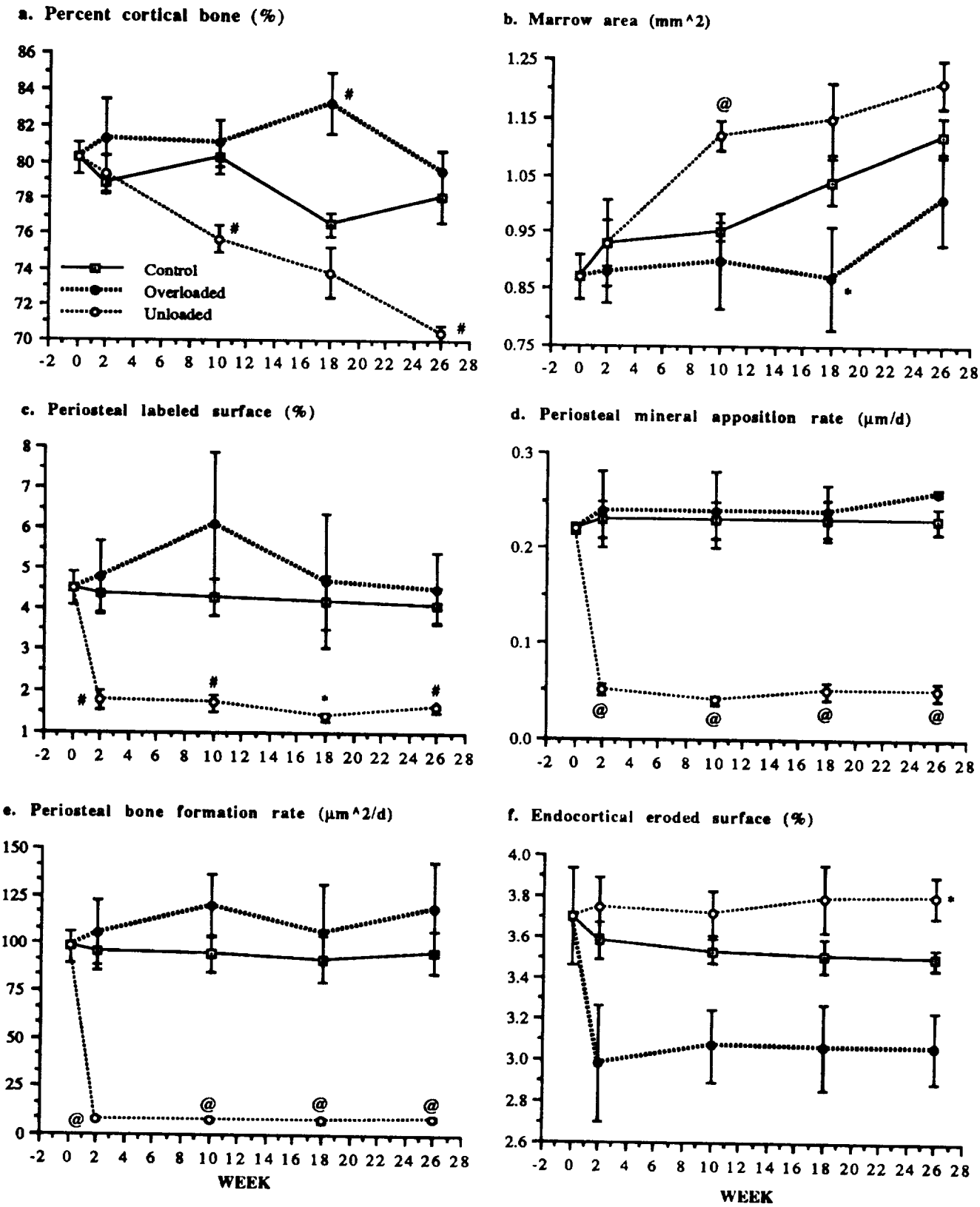

Fig. 5. Time course of static and dynamic histomorphometry changes of the tibial shaft in control, underloaded and overloaded limbs. Y error bars represent standard errors. $^{*}p < 0.5$, $^{\#}p < 0.1$ and $^{@}P < 0.001$ from controls. See text for explanation. Modified from Li and Jee, 1991; Jee *et al.*, 1991.

remodeling (resorption exceeding formation), respectively.

If bone remodeling dominates at the cancellous and endocortical envelopes, and only formation associated modeling (formation drift) exists at the periosteal envelope, and since underloading favors the resorption mode as discussed above, we can conclude that underloading decreases bone mass by stimulating bone resorption associated remodeling, and depressing bone formation associated modeling. In contrast, overloading increases bone mass by stimulating bone formation associated modeling and decreases bone resorption associated remodeling. In other words, while underloading stimulates bone remodeling-dependent bone loss and depresses bone modeling-dependent bone gain, overloading stimulates bone modeling-dependent bone gain and depresses bone remodeling dependent bone loss. Thus, there is general agreement with Frost's concept of skeletal adaptation to mechanical usage.

Advantages of The Rat Unilateral, Hindlimb Immobilization Model

One of the greatest advantages is that the rat is ideal for this type of work.

First, the rat is cheap, has restricted genetic variability, and can generate reproducible results.

Second, growth, modeling and remodeling dependent phenomena in cortical and cancellous bone can be studied independently in a single long bone.

Third, the rat immobilization model accurately models disuse osteopenia in humans. For human spinal cord injury, patients showed an average of 33% decrease in cancellous bone volume over 25 weeks and then stabilization after immobilization (Minaire *et al.*, 1974). Immobilization in these patients also caused an early increase in osteoclastic bone resorption and thinning of the cortices. These changes reflect a transient phase of bone loss, followed by a new steady state: rarefied bone with a lower rate of bone turnover. Further, investigators hypothesize an increase in the global life span of the bone remodeling unit (Minaire *et al.*, 1974, 1981; Vico *et al.*, 1987; Chappard *et al.*, 1989). We observe the same transient and rapid decrease in cancellous bone of -60% over 18 weeks and then stabilization, the early increase in bone resorption and decrease in bone formation and a reduction in cortical bone mass in our one-legged immobilized rat limbs. We confirm the existence of a transient state that leads to a new steady state. This new steady state is characterized by rarefied bone with a lower turnover rate. Furthermore, we confirm by histomorphometry that Minaire and his colleagues (1974) were correct in hypothesizing an increase in the sigma of the bone remodeling unit.

Our overloaded bone findings agree with Smith and Gulligan's (1989) report on adults under moderate overstrain; minimal periosteal response, possible intracortical response and formation greater than resorption. Our data also agree in general with Frost's model which states that increased mechanical usage decreases bone remodeling dependent bone loss and increases bone modeling dependent bone gain. However, the changes are small and would benefit from further careful studies.

Fourth, another advantage is that dynamic histomorphometry can be performed readily in rat bones if they are properly double-fluorescent labeled. Dynamic histomorphometry is a powerful tool and, to quote Parfitt (1990), can determine "the cumulative summation of all effects, whether primary or secondary, direct or indirect, immediate or remote; it is not merely a good way; it is, in the present state of knowledge, the only way".

Fifth, another advantage is that our unilateral hindlimb-immobilization model is unique in that both underloading and overloading can be studied in the same animal (Li *et al.*, 1990a; Li and Jee, 1991; Jee and Li, 1990; Jee *et al.*, 1991). The underloading findings are comparable to man and, surprisingly, the overloading was sufficient to add both cortical and cancellous bone. Whether this overloading model can add bone to both younger and older rats needs to be investigated.

Sixth, the final advantage is that the hindlimb bandaging model may be free of a regional acceleratory phenomenon (RAP) described by Frost, (1983b). A RAP is started by an injury or infection in a bone. It is a special reaction that accelerates all normal ongoing processes in the affected region. Injuries that can start a RAP include fractures, surgical operations, the insertion of devices, trauma and burns. Thus, surgical operated immobilization-induced osteopenia like tenotomy, amputation and neurectomy may mask the immobilization-induced osteopenia response. Jaworski and Uhthoff (1986) report that the initial, rapid phase of immobilization-induced bone loss by casting is from the RAP. They believe that the appearance of numerous resorption cavities and cavities that are larger than normal suggests not only a sudden increase in the number of activated sites, but also an increase in local recruitment of osteoclasts accounting for the larger resorption cavities. Apparently this response cannot be attributed solely to the RAP because the RAP only accelerates or intensifies on-going activities. It is questionable whether disuse-induced bone loss by bandaging or casting will evoked a RAP. These investigators did not supply any biochemical data that support there is an early RAP. Furthermore, immobilization-induced bone loss models like bed rest and weightlessness may not generate a noticeable RAP, but bedrest does provoke a marked increase of osteoclast numbers and resorption (Vico *et al.*, 1987; Chappard *et*

al., 1989). A slight, non-significant reduction in cancellous bone mass was observed in healthy men subjected to 120 days of bed rest compared to the massive loss of 1% per week in post trauma immobilized subjects (Minaire *et al.*, 1974; 1981). In another RAP-free model, 83 day old rats tested after 19 days of spaceflight exhibited decreased cancellous bone mass with inhibited bone formation and unchanged bone resorption (Jee *et al.*, 1983). Unfortunately, this is tainted data because it was generated several days after returning from the 19 day space flight so we are unsure whether elevated resorption did or did not occur. There is a dire need for information on tissue-level activities during weightlessness to determine the status of bone resorption. In both of these disuse-induced osteopenias, the conclusion that they are RAP-free and bone loss is due entirely to depressed bone formation cannot be dismissed. Whether disuse-induced bone loss by bandaging or plastic casting involves a RAP phase needs to be restudied.

Shortcomings of the Rat Rear One-legged Immobilization Model

Despite these advantages, there are some obvious shortcomings in our model as well. These include the small size of the bones, and the lack of osteonal remodeling in the rat.

First, the small bones in most rodents make it difficult to obtain biomechanical measurements. Surgical application of miniature strain gauges present a real problem (Keller and Spengler, 1982). There are mechanical properties reports available for cortical bone (Weir *et al.*, 1949; Saville and Smith, 1966; Engesaeter *et al.*, 1978; Kimura *et al.*, 1979; Vogel, 1979; Ekeland *et al.*, 1981, 1982a,b; Wunder *et al.*, 1979; Keller *et al.*, 1986), but not for cancellous bone.

The second disadvantage of this model is that the cortical bone in rats contains first generation Haversian systems which normally experience no secondary remodeling (Frost, 1973). Thus, an ideal site to determine the response of bone remodeling to mechanical usage is unavailable in rat cortical bone. However, a critical survey of data of large animals during disuse suggests that studying Haversian remodeling is not critical to understanding disuse-related cortical bone loss. Disuse-induced bone loss in the cortical bone of larger animals with Haversian remodeling is associated mostly with decreased periosteal formation and increased (compensatory surface remodeling of Martin and Burr, 1989) endosteal resorption, not defects in Haversian remodeling (Uhthoff and Jaworski, 1978; Jaworski *et al.*, 1980; Uhthoff *et al.*, 1985; Martin and Burr, 1989). The same mechanism acts in animals without Haversian remodeling (Li and Jee, 1991). **Thus, the lack of normal osteonal remodeling is not a real drawback of rat immobilization.**

These disadvantages are minimal. They can be overcome with care in the selection of an age group relevant to the type of question being asked. Even now the advantages vastly outweigh the disadvantages and the rat unilateral, rear bandaging model is the recommended model for pre-clinical evaluation of new therapies for the prevention and reversal of immobilization bone loss.

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Discussion with Reviewers

D. Chappard: A fatty involution of the bone marrow has been reported in spinal cord injured patients (Minaire). An increase of triglycerides has also been shown in the hind limb suspension model by Wronski and Morey. Did you find any difference of the adipose volume in their model between the two legs?

Authors: We did not quantify any fatty marrow changes, but qualitatively the amount of fatty marrow was higher in the unloaded proximal tibial metaphysis (PTM) and lower in the overloaded PTM compared to the controls.

D. Chappard: Simmons reported that overloading could increase the length of the bones of young rats. Although older rats were used in the present study, did the authors find an increased elongating rate in the overloaded leg?

Authors: These rats were much too old to enable us to measure proximal tibial bone elongation, but in the study of 2.5-month-old female rats, we observed to our surprise a transient depression of longitudinal growth after one and two weeks that stayed depressed for 20 weeks, the length of the study. At 8 weeks onward, this rate was comparable to age-related control level. We concluded that our model of overloading was unable to stimulate bone elongation.

D. Chappard: The trabecular number was found markedly reduced in this model (on the immobilized side) and increased in the overloaded side. This is in agreement with a rapid osteoclastic attack with perforating osteoclastic foci. The trabecular thickness, which has certainly been measured, should be interesting to discuss because the spatial rearrangement of the trabecular network is also an important factor contributing to the bone strength.

Authors: I don't know whether trabecular thickness is an important factor contributing to bone strength. Nor do I know of any published data. It is common sense that if you have thick trabeculae, they will persist longer. Thinner trabeculae will be more readily perforated and lost.

There was an obvious reduction in trabecular numbers by underloading and an increase in numbers by overloading compared to the age-related control. However, there was no increase in number compared to the beginning control. This means overloading maintained trabecular numbers, but never increased them. The increase in numbers by overloading when compared to control is because the controls lost trabeculae with age.

We need to better characterize the trabecular microstructure, the quantitation of modes and star volume are steps in the right direction. Also, some genius must find some way to measure cancellous bone strengths and how we can correlate trabecular thickness, numbers, etc., to strength. A good start would be the correlation of trabecular microstructure to the vertebral resistance to crushing.

R.B. Martin: How is minimodeling different from modeling? Does minimodeling include resorption as well as formation modes?

Authors: Minimodeling is a work coined by Harold Frost for modeling on a smaller scale involving trabeculae. The drifting of osteons may be classified as such. It adheres to all the mode of action of classical modeling.

R.B. Martin: Is it feasible to discriminate between modeling and remodeling in the rat model as Frost originally did, by documenting the existence of cement lines beneath bone forming surfaces? If so, cannot this be done in the tibial metaphysis, as Baron *et al.*, (1984) have done for the tail vertebrae of the rat?

Authors: We have not been able to use Hal Frost's method of classifying remodeling and modeling cement lines in our material. The problem may be that most Howship's lacunae in the rat are quite shallow and lacking in scalloped reversal lines. We should try to get Dr. Frost involved in such an exercise. Also, we have a difficult time producing sections that are wrinkle- and crack-free thin sections and well stained for cement lines. Baron *et al.* (1984) have done it for caudal vertebrae, but few of us can do it for the proximal tibia. Finally, there should be some agreement on the occurrence of modeling in the adult skeleton before one can proceed. Parfitt's (1983, 1990) description of modeling pertains to the growing rather than the adult skeleton.

H.M. Frost: I would like to comment on three important aspects of your studies. First, these studies carried the experimental challenge on long enough to achieve steady states, whereas many other people interested in similar matters failed to do that. Because of that, relatively small mechanical usage effects that could not be measured accurately after periods of a month or two, have time enough to become large enough to exceed the precision and errors of measurement. Second, the present authors have quite properly considered the unimmobilized leg to be mildly overloaded instead of normal, unlike most others who studied similar animal models. Providing proper controls then brought out the fact that this mild overloading did indeed lead to increased accumulations of cortical and trabecular bone. Third, the authors have distinguished bone modeling due to drifts, from BMU-based remodeling due to basic multi-cellular units, and designed their experiments in such a way as to show the effects of mechanical under and overloading on those activities separately. Again, a majority of others who have published animal experiments dealing with this problem failed to do that. Do you plan to repeat these studies in rapidly growing rats?

Authors: We have just completed a similar study beginning with 2.5 -month-old rats and are in the middle of an experiment beginning with weanling rats.

D.B. Kimmel: Most people have found that the rats struggled free from the elastic bandage taping within several hours or by the next day. Was the rats chewing the tape a problem? Did you do daily monitoring? Did you use cages with bedding in the bottom? These are little hints that will help others to understand how you were successful where most have failed with this model.

Authors: We monitored the rats daily to see that the elastic bandage and the immobilized leg were in place. If there were signs of chewing to the point there was any question that the leg would remain in place, the bandage was replaced. This rebandaging took place every two to three days. It makes for a time-consuming, but successful experiment. With this continual checking and taping, the rats quickly become accustomed to the procedure. All this is done with two people without anesthesia very quickly and easily.

We bed our animals in commercial corn cob bedding.

D.B. Kimmel: Do you have any comments on how the animals adapt to the confinement during the first several weeks? Is there stress and weight loss due to varied eating patterns?

Authors: The animals adapt quite readily and easily to the confinement. Within 24 hours, young animals are carrying all their weight on three legs as if they never had a fourth. Older animals take a little longer to adapt to getting around on three legs. We put a little food in the bottom of the cage during the adjustment period. There is loss of "body fat" and hair loss in the waist area of the bandaging with the initial taping. Perhaps this is due to sweating under the wrap. In these experiments with the older rats, there was no difference in body weights between the local immobilized and age-matched controls at autopsy (Li *et al.*, 1990a). The animals are able to move freely around the cage, climbing on the wire tops, and standing up to drink; this type of immobilization allows them to return to their normal routine, thus minimizing stress.

D.B. Kimmel: For a bit of background, do we know anything about the acute response of muscle to disuse?

Authors: We routinely weigh the gastrocnemius muscle at autopsy, but did not perform anything more fancy and there is an extensive bibliography on immobilization-induced muscle loss. There is an early drop in muscle weight that stabilizes at approximately 50% of control level. Please also see Li *et al.*, 1990a. Please refer to several references included in the text for further details.

D.B. Kimmel: I agree that one cannot rule out that "[disuse-associated] bone loss is due entirely to depressed bone formation." In fact, it might be the predominant mode. That would explain the difference in growing and adult animals. Growing ones with lots of modeling (an activity for increasing [strength] and mass), respond quickly with profound decreases in mass [or failures to gain]. Adult ones with modeling, respond slowly with more minor decreases in mass. It begins to sound like the corticosteroid story. Our uncertainties for measuring resorption may have again caused us to speculate about its status when the proper conclusion is that we (frustratingly) don't know!

Authors: We agree with your statement on the uncertainties for measuring resorption. However, we inserted the sentence on "(disuse-associated) bone loss is due entirely to depressed bone formation" to be provocative. We cannot overlook the fact that the early immobilized-induced stimulated bone resorption eliminates a lot of bone surface, thus wiping out available bone surfaces for bone formation. In other words, it uncoupled bone formation from resorption because of the lack of bone surface. We believe the lack of bone surface along with the direct depression of osteoblasts is the cause of depressed bone formation. Finally, we agree that older skeleton loses bone slower, but both the growing (unpublished data) and older rats at steady state have lost about the same amount of bone.